Human placental vascular reactivity in health and disease: implications for the treatment of preeclampsia

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ABSTRACT

Adequate development of the placenta is essential for optimal pregnancy outcome. Pre-eclampsia (PE) is increasingly recognized to be a consequence of placental dysfunction and can cause serious maternal and fetal complications during pregnancy. Furthermore, PE increases the risk of neonatal problems and has been shown to be a risk factor for cardiovascular disease of the mother later in life. Currently, there is no adequate treatment for PE, mainly because its multifactorial pathophysiology remains incompletely understood. It originates in early pregnancy with abnormal placentation and involves a cascade of dysregulated systems in the placental vasculature. To investigate therapeutic strategies it is essential to understand the regulation of vascular reactivity and remodeling of blood vessels in the placenta. Techniques using human tissue such as the *ex vivo* placental perfusion model provide insight in the vasoactive profile of the placenta, and are essential to study the effects of drugs on the fetal vasculature. This review highlights the different pathways that are involved in the vascular regulation of the human placenta, changes that occur during PE and the importance of focusing on restoring these dysfunctional systems when studying treatment strategies for PE.

INTRODUCTION

The placenta is an essential regulatory organ that provides the fetus with nutrients and oxygen, determines the passage of pharmacological and toxic agents, and regulates the endocrine and immune systems.[1] Optimal placental function is crucial for fetal health and subsequent neonatal outcome. Insufficient development of the placenta is increasingly recognized to underlie serious pregnancy complications such as preeclampsia (PE) and fetal growth restriction (FGR), thereby contributing to both maternal and perinatal morbidity and mortality.[2-4]

PE is a multi-system disorder, clinically characterized by de novo onset hypertension and proteinuria after 20 weeks of gestation, which complicates approximately 3-8% of pregnancies.[3] Two forms of PE can be distinguished: early onset PE, manifesting before the 34th week of gestation, and late onset PE, manifesting after the 34th week of gestation.[5] PE can have serious maternal consequences, such as kidney failure, liver disease, cerebral hemorrhage and lung edema, but can also result in FGR and/or premature birth. Evidence is accumulating that placenta-related pregnancy complications increase the risk of maternal and neonatal health problems in later life. For example, women who have suffered from PE have a higher chance of developing cardiovascular diseases,[6, 7] and children born prematurely or with low birth weight have an increased risk of impaired cardiopulmonary and neurological development.[8-10] Currently, there is no appropriate therapy available for PE. Treatment is aimed at prolonging pregnancy by symptom relief and prevention of further complications, but the only cure is delivery. However, this is often harmful for the preterm fetus, and therefore development of novel treatment options to safely prolong pregnancy is very important.

Although the exact etiology of PE remains unknown, a large body of evidence indicates that it originates in the first weeks of pregnancy and is the result of abnormal placentation. Initially, impaired trophoblast invasion leads to aberrant remodeling of the spiral arteries, resulting in higher placental vascular resistance and hypo-perfusion, with oxidative stress related to ischemia-reperfusion damage and increased placental production of anti-angiogenic factors. The combination of these factors induces generalized vascular dysfunction, thereby contributing to the hypertension and proteinuria occurring in PE patients.[11-13] Since early- and late onset PE show distinct histopathological differences,[5] separate pathogenic pathways have been suggested, proposing that late onset PE might be a predominant maternal syndrome, rather than a placental disorder.[14, 15]

Many vasoactive pathways have been proposed to be altered in PE, although data on the human placental vasculature are largely inconclusive. To investigate therapeutic strategies it is essential to understand the regulation of vascular tone and remodeling of blood vessels in the healthy placenta, and changes in PE. Research using the *ex vivo*

placental perfusion system or wire-myography, two models to study human tissue, may provide insight into vasoactive characteristics of the placenta. *Ex vivo* dual-sided placental perfusion is an experimental model to study vascular reactivity of the fetal side of the placenta in a single cotyledon, and using wire-myography similar experiments can be performed in isolated vessel segments.

The purpose of this review is to summarize the vascular reactivity profile of the fetal side of the human placenta, by describing its most important pathways and factors, being vascular endothelial growth factor (VEGF), the endothelin (ET) system, the renin-angiotensin system (RAS), prostaglandins, nitric oxide (NO) and NO-dependent vasodilators, serotonin and tryptophan (Trp), and calcitonin gene-related peptide (CGRP). Relevant studies were identified by making use of a systematic search in literature on 20 April 2018. We highlight changes that occur during, and/or contribute to the pathophysiology of PE, and discuss possible treatment strategies based on interfering with the regulation of placental vascular tone.

DEVELOPMENT OF THE FETOPLACENTAL VASCULATURE

Adequate development of the placental vasculature is essential for normal growth and development of the fetus during pregnancy.[1] Many pregnancy complications are associated with disturbed placentation, such as PE, FGR, preterm birth and spontaneous abortion.[16] At term, the placenta consists of cotyledons, i.e., villous trees that are supplied by arteries branching off the umbilical circulation. These are surrounded by intervillous space filled with maternal blood coming from spiral arteries, to enable the exchange of oxygen and nutrients between mother and fetus.[1]

During embryonic implantation, the outer layer of the blastocyst, the trophectoderm, invades deep into the uterine wall. The trophectoderm differentiates into multiple types of trophoblast cells, including cytotrophoblast cells and, when these cells fuse, syncytiotrophoblast cells.[17] The process of differentiation is closely regulated by multiple growth factors, hormones and environmental factors such as oxygen tension.[18] The syncytiotrophoblast layer covers the cytotrophoblast and lines the villous trees, making direct contact with the maternal circulation and thereby playing an important role in the supply of oxygen and nutrients from mother to fetus, and waste products and carbon dioxide from fetus to mother. Furthermore, these cells are involved in pregnancy-related hormone production.[17] In early gestation, when there is no direct exchange of oxygen and nutrients between the fetal and maternal circulations, the embryo is provided with nutrients via diffusion from the decidua.[19] At this stage, cytotrophoblast plugs occlude the spiral arteries, allowing diffusion but preventing perfusion of the intervillous space, this way keeping a low oxygen environment.[20] Around 10 weeks of gestation, extravillous

cytotrophoblast cells invade around the spiral arteries, initiating their remodeling and unplugging. This remodeling encompasses a 5- to 10-fold increase in terminal lumen diameter and structural changes of the vessel wall, such as demuscularization, creating a low resistance circulation.[12, 18] Because of these changes, the perfusion capacity is increased and the blood velocity into the intervillous space is reduced to protect the vulnerable villous tree and to optimize exchange of oxygen and waste products.[12] After the vascular network has been formed in early pregnancy, capillary growth continues until delivery, mediated by various growth factors. From mid-gestation onwards, there is an exponential growth in vascular volume of placental vessels to accommodate the needs of the growing fetus.[1] Unlike most other blood vessels, vessels of the fetoplacental circulation are not innervated. Local vascular tone and fetal cardiac output are the main determinants of the blood flow through these vessels, regulated by circulating and locally produced hormones and vasoactive compounds, such as estrogen, prostaglandins, ET-1 and NO.[21] The most important pathways that are involved in the regulation of placental vascular tone and changes occurring in PE, as will be discussed in this review, are summarized in Table 1. Drugs targeting these systems and their potential relevance in the treatment of PE are described in Table 2.

VASCULAR ENDOTHELIAL GROWTH FACTOR

The VEGF pathway plays a pivotal role in the formation and development of blood vessels during growth of the human placenta.[22] The VEGF system has not only been implicated in the promotion of angiogenesis, but is also crucial to maintain vascular endothelial function. VEGF induces the release of NO and prostacyclin (PGI_2) from endothelial cells, indicating its potential in the regulation of vascular tone (Figure 1).[23, 24] VEGF acts in a paracrine manner on endothelial cells, and its expression has been localized to villous trophoblast cells, maternal and fetal macrophages, decidual cells and the fetal membranes of the placenta.[23, 25] Its biological actions are elicited upon binding to tyrosine kinase receptors (VEGFRs), of which VEGFR-1 and VEGFR-2 are considered the main functional receptors in the placenta. VEGFR-2 plays a major role in angiogenesis through promoting endothelial cell proliferation and vascular formation. Its location is restricted to fetal endothelial cells and syncytiotrophoblasts.[26] VEGFR-1 is expressed in fetal and umbilical vein endothelial cells, decidual cells and (extra)villous trophoblast cells.[27] While the function of VEGFR-1 is not entirely understood, it is thought that it regulates angiogenesis either positively or negatively through mechanisms involving VEGF-trapping and homo- or heterodimerization with VEGFR-2.[28-30] Placental growth factor (PlGF), which is homologous to VEGF, is considered equally important in the regulation of placental angiogenesis, through its interaction with VEGFR-1.[23] In addition,

Table 1. Vascular reactivity pathways in the human placenta and changes occurring during preeclampsia.

Pathway	Function in normal pregnancy	Changes in maternal plasma during PE	Changes in placental tissue during PE
VEGF	Promotion of angiogenesis; Maintaining vascular endothelial function; Stimulating release of NO and PGI_2	Decreased levels of VEGF and PlGF; Increased levels of sFlt-1	Increased gene expression of VEGF; No change in PlGF gene expression; Increased gene expression of sFlt-1
Endothelin	Vasoconstriction; Promotion of trophoblast proliferation and invasion; Initiation of uterine contractions	Increased levels of ET-1, MMPs and ECE	Increased gene expression of ET-1 and MMP-2; Decreased gene expression of ET_A receptor; Increased/decreased gene expression of ET_B receptor
RAS	Regulation of vascular tone;	Decreased levels of renin, Ang I, Ang II and aldosterone; Increased Ang II sensitivity;	Increased levels of angiotensinogen and Ang II;
	Sodium homeostasis	Increased levels of AT_1R-AA; Decreased AT_2R expression	Increased expression of AT_1R; Upregulation of ACE and chymase
Prostaglandins	Vasoconstriction (TxA_2, PGE_2, $PGF_{2\alpha}$); Stimulation of platelet aggregation and uterine contractility (TxA_2); Vasodilation and inhibition of platelet aggregation and uterine contractility (PGI_2)	Increased levels of TxA_2 and lipid peroxides; Decreased levels of PGI_2	Increased production of TxA_2; Reduces release of PGI_2; Increased expression of COX-1
Nitric oxide	Vasodilation	Decreased levels of NO metabolites and NO-mediated vasodilators; Increased levels of ADMA	Increased gene expression of eNOS Increased levels of peroxynitrite
Bradykinin	Vasodilation through stimulation of NO and PGI_2 release; Vasoconstriction through stimulation of TxA_2 production; Stimulation of cell migration and trophoblast invasion		Reduction of gene - and protein expression of B2 receptor
Acetylcholine	Influencing placental transfer of amino acids; Influencing placental hormone release		Decreased synthesis of acetylcholine; Decreased density of mAChR; Increased expression of nAChR

Table 1. Vascular reactivity pathways in the human placenta and changes occurring during preeclampsia. (continued)

Pathway	Function in normal pregnancy	Changes in maternal plasma during PE	Changes in placental tissue during PE
Histamine	Promotion of trophoblast proliferation and invasion; Vasoconstriction (through H1 receptor); Vasodilation (through H2 receptor)		Increased tissue concentrations of histamine; Higher mast cell density; Reduced sensitivity to histamine
Serotonin	Vasoconstriction	Increased levels of serotonin	Increased sensitivity to serotonin in microvasculature
Tryptophan	Vasodilation through metabolism by IDO1		Reduced expression and activity of IDO1
CGRP	Influencing vascular adaption; Maintaining uterine relaxation during pregnancy; Vasodilation	Decreased levels of CGRP	Reduced mRNA and protein expression of CRLR and $RAMP_1$

Abbreviations: ACE = angiotensin converting enzyme; ADMA = asymmetric dimethylarginine; Ang I = angiotensin I; Ang II = angiotensin II, AT_1R = angiotensin II type 1 receptor; AT_1R-AA = angiotensin II type 1 receptor auto-antibodies; AT_2R = angiotensin II type 2 receptor; B2 = bradykinin receptor; CGRP = calcitonin gene-related peptide; COX-1 = cyclooxygenase-1; CRLR = calcitonin receptor-like receptor; ECE = endothelin converting enzyme; eNOS = endothelial nitric oxide synthase; ET-1 = endothelin-1; ET_A = endothelin-1 type A; ET_B = endothelin-1 type B; H1 = histamine type 1; H2 = histamine type 2; IDO1 = indolamine 2,3-dioxygenase; mAChR = muscarinic acetylcholine receptor; MMP = matrix metalloproteinase; nAChR = nicotinic acetylcholine receptor; NO = nitric oxide; PE = preeclampsia; PGI_2 = prostacyclin; PlGF = placental growth factor; $RAMP_1$ = receptor activity modifying protein-1; sFlt-1 = soluble Fms-like tyrosine kinase-1; TxA_2 = thromboxane A_2; VEGF = vascular endothelial growth factor.

Table 2. (Potential) Treatment strategies to target the dysfunctional placental vascular reactivity in preeclampsia.

Target	Therapy	Mechanism of action	Preclinical studies	Clinical trials
VEGF	Recombinant VEGF	Supplementation of VEGF	Attenuated hypertension in rats	NA
	Relaxin	Upregulation of VEGF	Attenuated hypertension in rats	NA
sFlt-1	Dextrane sulphate apheresis	Extracorporeal removal of sFlt-1	Absorbed recombinant sFlt-1 in human whole blood	Alleviated PE symptoms
	HO-1	Inhibition of sFlt-1 release	Attenuated hypertension in rats	NA
	Proton pump inhibitors	Upregulation of HO-1	Inhibited sFlt-1 secretion in placental explants	No effect on PE symptoms
	Statins	Upregulation of HO-1	Inhibited sFlt-1 secretion in placental explants	NA
	Ouabain	Downregulation of placental sFlt-1 production	Inhibited sFlt-1 secretion in placental explants and human trophoblast	NA
	Metformin	Downregulation of placental sFlt-1 production	Inhibited sFlt-1 secretion in placental explants and primary human tissue	Reduced incidence of PE
PlGF	Administration of PlGF	Supplementation of PlGF	Attenuated hypertension and proteinuria in rats	NA
Endothelin	ERAs	Blocking ET-1 receptors	Attenuated hypertension and proteinuria in rats	NA
	small interfering RNA	Silencing sFlt-1 mRNA	Attenuated PE symptoms in baboons	NA
RAS	ACE inhibitors	Inhibition of the conversion of Ang I into Ang II	Increased risk of IUFD and stillbirth	Teratogenic effects on fetus
	AT receptor blockers	Blocking the AT_1R	Increased risk of IUFD and stillbirth	Teratogenic effects on fetus
	n7AAc	AT_1R-AA antagonism	Attenuated hypertension and reduced sFlt-1 and preproET-1 levels	NA
Prostaglandins	Low dose acetylsalicylic acid	Inhibition of TxA_2 synthesis through COX-inhibition		Reduced the risk of developing PE in high risk patients
	Celecoxib	Selective COX-2 inhibition	Attenuated hypertension and improved fetal growth	NA

Table 2. (Potential) Treatment strategies to target the dysfunctional placental vascular reactivity in preeclampsia. (continued)

Target	Therapy	Mechanism of action	Preclinical studies	Clinical trials
Nitric oxide	Organic nitrates/ S-nitrosothiols	Exogenous NO-donors		Decrease in blood pressure, no effect on maternal or fetal outcome and significant side-effects
	N-acetylcysteine	Antioxidant	Improved NO-mediated vasodilation in fetoplacental vasculature	No improvement in severe early onset PE
	YC-1/Riociguat	sGC activation or stimulation	Inhibited sFlt-1 production and endothelial dysfunction in PE tissue	NA
	Sildenafil	Reducing cGMP degradation through PDE5 inhibition	Reduced maternal PE symptoms and improved fetal outcome	Halted due to lack of beneficial effects and a possible increase of neonatal complications
Bradykinin	ACE inhibitors	Inhibition of BK degradation	Increased risk of IUFD and stillbirth	Teratogenic effects on fetus
Acetylcholine	Nicotine	Stimulation of the nAChR	Stimulated production of VEGF and PlGF	NA
Histamine	H1-antagonist	Inhibition of vasoconstriction	NA	NA
	H2-agonist	Stimulation of vasodilation	NA	NA
Serotonin	Ketanserin	5-HT2 receptor antagonist	Decreased blood pressure and placental blood flow in hypertensive rats	Led to persistent hypertension and there was no beneficial effect on pregnancy outcome
CGRP	Administration of CGRP	Supplementation of CGRP shortage	Reduced maternal hypertension and pup mortality in rats	NA
	Rutaecarpine	Potentiation of endogenous CGRP release	Reduced blood pressure in hypertensive rats	NA
	αCGRP analogue	Supplementation of CGRP shortage	Antihypertensive effects in cardiovascular murine studies	NA

Abbreviations: 5-HT2 = 5-hydroxytryptamine-2; ACE = angiotensin converting enzyme; Ang I = angiotensin I; Ang II = angiotensin II, AT_1R = angiotensin II type 1 receptor; AT_1R-AA = angiotensin II type 1 receptor auto-antibodies; CGRP = calcitonin gene-related peptide; COX = cyclooxygenase; ERA = endothelin receptor antagonist; ET-1 = endothelin-1; H1 = histamine type 1; H2 = histamine type 2; HO-1 = heme-oxygenase-1; IUFD = intrauterine fetal death; nAChR = nicotinic acetylcholine receptor; NA = not applicable; NO = nitric oxide; PDE5 = phosphodiesterase-5; PE = preeclampsia; PGI_2 = prostacyclin; PlGF = placental growth factor; sFlt-1 = soluble Fms-like tyrosine kinase-1; sGC = soluble guanylate cyclase; TxA_2 = thromboxane A_2; VEGF = vascular endothelial growth factor.

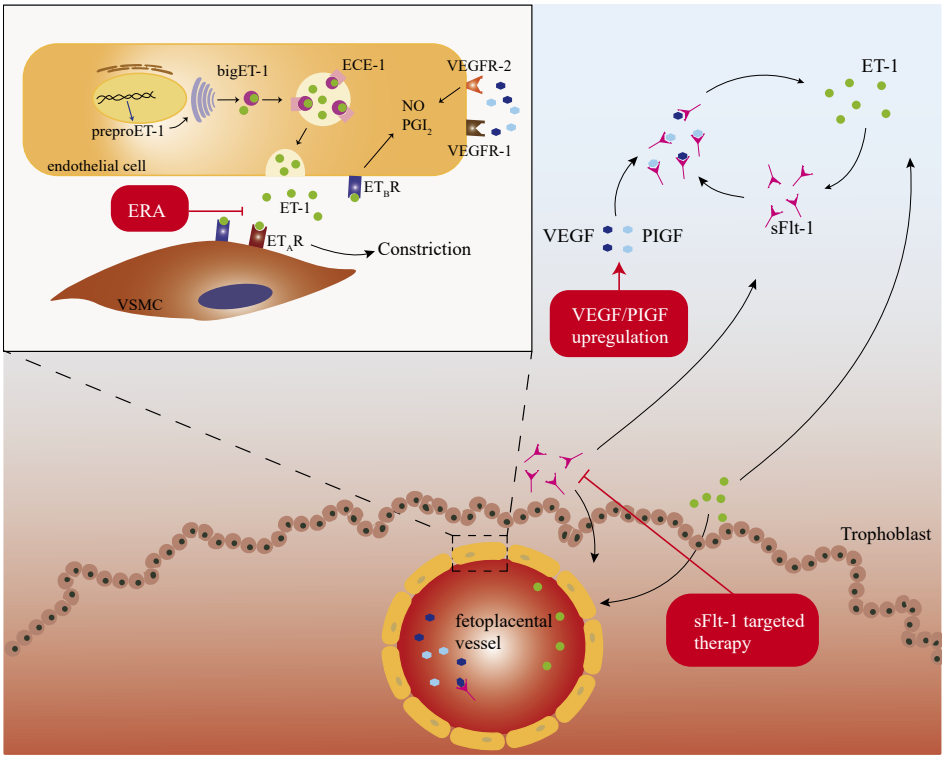

Figure 1. VEGF and ET-1 system in the human placenta and therapeutic strategies targeting this pathway. During PE there is an increase in sFlt-1 and ET-1, whereas VEGF and PlGF are decreased. Abbreviations: bigET-1 = big-endothelin-1; BM = basal membrane; ECE-1 = endothelin converting enzyme-1; ERA = endothelin receptor antagonist; ET-1 = endothelin-1; ET_AR = endothelin type A receptor; ET_BR = endothelin type B receptor; NO = nitric oxide; PGI_2 = prostacyclin; PlGF = placental growth factor; preproET-1 = preproendothelin-1; sFlt-1 = soluble Fms-like tyrosine kinase-1; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor; VSMC = vascular smooth muscle cell.

soluble Fms-like tyrosine kinase (sFlt-1), a soluble form of VEGFR-1 generated through alternative splicing, binds with high affinity to its natural ligands VEGF and PlGF, thereby preventing interaction with their receptors (Figure 1).[23] It is produced and released into the maternal circulation by villous trophoblast cells of the placenta.[31] Interestingly, sFlt-1 levels found in the venous perfusate of the maternal side in the perfused cotyledon of healthy human placentas closely reflect *in vivo* serum values, indicating that sFlt-1 is predominantly secreted by the placenta.[27] Moreover, Saleh *et al.* observed a rapid postpartum decline by >99% in circulating sFlt-1 levels of women with suspected or confirmed PE, further suggesting the placenta is the major source of sFlt-1 secretion.[32]

In healthy pregnancy, VEGF is highly expressed by placental tissue during the first trimester[13] and is thought to act as a chemoattractant promoting trophoblast invasion.[23] In contrast, circulating sFlt-1 levels are relatively low in early pregnancy and begin to rise

during the third trimester, reflecting an antiangiogenic shift toward the end of pregnancy.[13] Excess sFlt-1 produced by preeclamptic villous tissue attenuates fetoplacental angiogenesis *in vitro*,[33] implicating that the physiological rise in sFlt-1 levels during healthy pregnancy is essential for adequate control of placental angiogenesis. Studies examining the release of VEGF in the *ex vivo* perfused cotyledon of term placentas are consistent with the former findings, showing a bilateral release of total VEGF, predominantly in the maternal circulation, with a high ratio of sFlt-1 to free VEGF, resulting in near complete VEGF binding. Interestingly, free VEGF levels were only detected on the fetal side. This finding may not be too surprising, as sFlt-1 levels are marginally secreted on this side, so that free VEGF will not be sequestered.[27] In the fetoplacental vasculature, VEGF acts as a potent vasodilator via the interaction with VEGFR-2. *Ex vivo* administration of free VEGF to the fetal side of the perfused cotyledon after pre-constriction with U46619 caused a dose-dependent vasodilation in the fetal circulation, which was partially attenuated by NO synthase inhibition.[27] Similar observations were made in isolated human chorionic plate arteries; VEGF evoked a concentration-dependent vasodilator response in the fetal circulation following pre-constriction with phenylephrine.[24] Importantly, the concentrations that elicit a vasodilator response in the fetoplacental vasculature are within the physiological range of fetal serum levels of VEGF. With regard to PlGF, no changes in fetal perfusion pressure were observed in the fetoplacental vasculature, while it significantly provoked vasodilation in isolated human chorionic plate arteries.[24, 27] These apparent opposite findings suggest that large arteries contribute relatively little to total resistance of the placental vasculature.

In recent years, excessive placental production of sFlt-1 has been implicated to be involved in the pathogenesis of PE. Multiple studies have reported elevated sFlt-1 levels in the circulation along with decreased free VEGF and free PlGF levels in women with PE,[34, 35] while PE placentas displayed increased expression of sFlt-1 mRNA.[36] In pregnant mice, sFlt-1 infusion or injection of an adenovirus encoding the sFlt-1 gene, elicits the hallmark features of PE, including hypertension, proteinuria and glomerular endotheliosis.[36, 37] Furthermore, treatment of cancer patients with VEGF antagonists induces a PE-like syndrome, with hypertension and proteinuria.[38] Consequently, reduced circulating levels of free VEGF and free PlGF promote the maternal syndrome of PE. Moreover, significant alterations of the VEGF system have been implicated in the fetoplacental vasculature during PE, which may actively contribute to increased blood flow resistance in the placental circulation.[27, 39] The increase in placental sFlt-1 production may be triggered by several factors during PE, including inflammatory cytokines, angiotensin II type 1 receptor auto-antibodies (AT_1R-AA) and placental hypoxia, most likely involving hypoxia inducible factor-1α (HIF-1α) as a mediator.[40, 41] Subsequently, sFlt-1 mediates hypertension through impairing endothelial NO production, which is normally upregulated by VEGF, thereby inducing endothelial dysfunction.[42] This is accompanied by upregulated

ET-1 production,[43] most likely as a consequence of impaired inhibition of ET-production by NO, and further reflecting endothelial dysfunction. Brownbill *et al*., making use of the *ex vivo* dual-sided placenta perfusion model, observed an enhanced vasodilator effect in the fetal circulation in response to exogenous VEGF administration in PE placentas as compared with healthy controls.[39] However, this response was only seen at high VEGF doses, which fall outside the physiological range of fetal serum concentrations. Moreover, diminished fetal serum concentrations of free VEGF were reported, in accordance with higher fetal sFlt-1 levels during PE, probably contributing to a reduced vasodilator response of the fetoplacental vasculature *in vivo*. Where the higher fetal sFlt-1 levels arise from is largely unknown, although one could hypothesize that the trophoblasts either secrete more sFlt-1 into the fetal circulation, or that the excessive maternal sFlt-1 levels are, at least in part, transported to the fetal side. Notably, an important role for VEGF localized to decidual tissue of the placenta has been identified. Fan *et al*. reported that endometrial-specific VEGF overexpression in mice significantly induced sFlt-1 release.[44] Combined with the increased decidual expression of VEGF mRNA in human PE placentas, this observation may indicate that VEGF regulates sFlt-1 release in the placenta at the maternal-fetal interface.[44]

Several studies have focused on improving the angiogenic imbalance in PE, for instance, through the extracorporeal removal of sFlt-1 using dextrane sulphate apheresis.[45] This resulted in significant alleviation of PE symptoms.[45] Furthermore, treatment with recombinant VEGF both *in vitro* and *in vivo* partially reduced the antiangiogenic consequences of excess sFlt-1 in an animal study.[46] With regard to the fetoplacental vasculature, administration of recombinant VEGF might aid in reducing placental vascular resistance during PE. However, concern has been raised regarding potential harm to the fetus by VEGF that passes the placental barrier. The recent development of VEGF fused to elastin-like polypeptide, a biopolymer carrier that does not cross the placental barrier, could overcome this problem.[46] Similarly, administration of PlGF in a PE rat model has shown promising results in reducing hypertension and proteinuria,[47] while there are no studies assessing the vasoreactive effects of PlGF in the fetoplacental vasculature during PE. Another potential target for improving the VEGF pathway may be through the vasodilator hormone relaxin, a local upregulator of VEGF, that could potentially improve fetoplacental blood flow during PE.[31] The safety profile of relaxin is currently being investigated for the treatment of women with PE.[31]

Inhibition of sFlt-1 secretion in the placenta itself forms an ideal approach towards restoring the angiogenic imbalance and improving placental blood flow. Moreover, given that sFlt-1 induces a rise in ET-1, such treatment would be able to target the ET-1 system. Some may argue that this method is not desirable, since sFlt-1 appears to be required to maintain placental health.[45] Several research groups have focused on inhibiting the release of sFlt-1 upstream. For instance, heme-oxygenase-1 (HO-1) attenuated

sFlt-1 induced hypertension *in vivo*.[48] Onda *et al.* observed decreased sFlt-1 secretion from placental explants treated with proton pump inhibitors (PPI), which are known to upregulate HO-1.[49] In keeping with this data, our group has found lower sFlt-1 and ET-1 levels in women with (suspected) PE that were on PPI treatment.[50] Recently, Cluver *et al.* conducted a randomized controlled trial involving 119 women with PE, and found no difference in median sFlt-1 levels between women treated with PPI and women treated with placebo.[51] These conflicting findings demand future studies to establish the role of PPI treatment on sFlt-1 in women with PE. Statins also inhibit sFlt-1 secretion in placental explants through the stimulation of HO-1.[52] While the safety profile of the latter is still under investigation, PPIs have been proven safe during pregnancy.[51, 53] Ouabain, a cardiac glycoside, downregulates placental sFlt-1 production *in vitro* through inhibition of the HIF-1α pathway, while similar effects were observed with metformin, possibly by blockade of the mitochondrial electron transport chain.[54, 55] Several other agents may be under evaluation for their potential sFlt-1 inhibiting properties, however the safety of their use during human pregnancy remains a main point of concern.

In summary, PE is characterized by increased circulating levels of sFlt-1, and decreased levels of VEGF and PlGF, causing an anti-angiogenic imbalance. Restoring this imbalance showed promising results in animal studies, and clinical trials are now awaited.

ENDOTHELIN

Over the last 3 decades, a dominant role for the ET system has emerged in the modulation of vascular tone in many organ tissues, including the human placenta. ETs belong to a family of three 21-amino acid peptides (ET-1, ET-2, ET-3), with ET-1 being the predominant vascular isoform and currently one of the most potent vasoconstrictors known.[56] ET-1 is secreted by endothelial cells and trophoblast cells towards the basolateral side of these cells, indicating its role as a paracrine or autocrine peptide.[57, 58]

Derived from its precursor peptide known as preproendothelin-1 (preproET-1), big-endothelin-1 (bigET-1) is cleaved by endothelin converting enzymes (ECEs) and other enzymes, including matrix metalloproteinases (MMP) and chymase, into biologically active ET-1.[59] Once synthesized, ET-1 modulates vascular tone through two cell surface G-protein-coupled receptors; ET type A (ET_A) receptors located on the vascular smooth muscle cell (VSMC) and ET type B (ET_B) receptors located on endothelial cells and VSMCs (Figure 1). Whereas ET_A receptors and ET_B receptors on VSMCs both induce vasoconstriction, ET_B receptors on endothelial cells account for the vasodilator effects of ET-1 through the release of NO and prostaglandins, and additionally provide the means for clearing ET-1 from the circulation.[59, 60] Receptor localization studies have localized ET_A receptors in the veins and arteries of the chorionic plate, while ET_B receptors were identi-

fied in decidual cells, veins in stem villi, and blood vessels in distal regions of the villous tree.[61] ET-binding sites have also been localized in trophoblastic tissue.[62] Stimuli for ET-1 release include ET-1 itself, hypoxia, inflammatory cytokines, along with other vasoactive substances such as Ang II.[59]

The functional role of the ET system in healthy pregnant women is not fully understood. ET-1 has been demonstrated to exert mitogenic effects, promoting first trimester trophoblast proliferation and invasion *in vitro*.[63] In addition, expression studies have shown an increased ratio of ET_B to ET_A receptors in the human placenta of healthy pregnant women compared to other vessel beds, which may account for the enhanced local vasodilatory state during pregnancy.[64] While maternal serum levels of ET-1 remain similar to those of non-pregnant women during gestation, a substantial rise is detected during labor.[65, 66] Increased uterine expression of ET_A and ET_B receptors was also observed during parturition, implicating ET-1 is involved in the initiation of uterine contractions.[67] High concentrations of ET-1 were also found in amniotic fluid and the fetal circulation including the umbilical vessels, being almost three-fold higher in the umbilical vein when compared to maternal serum levels during labor.[68] Furthermore, ET-1 was found to be a potent vasoconstrictor of human umbilical vessels.[69] Subsequently, earlier studies hypothesized that ET-1 is an important regulator of placental vascular tone. Wilkes *et al.* were the first to report a dose-dependent increase in perfusion pressure caused by ET-1 in the *ex vivo* perfusion model,[70] implying vasoconstriction. Furthermore, a long-lasting, strong concentration-dependent vasoconstrictor response to ET-1 was observed in isolated chorionic plate vessels, stem villous and umbilical vessels, albeit to a higher degree in veins than in arteries.[71, 72] However, blockade of the ET_A receptor in placental veins resulted in little inhibitory effect on ET-1 mediated contractions, implying a high abundance of constrictor ET_B receptors in placental veins.[73] Hence, differences in receptor distribution could account for the increased ET-1 sensitivity in placental veins, and may contribute to different vasopressor responses within the placenta. In placental arteries, the contractile response to ET-1 was significantly abolished by blocking ET_A and to a lesser degree ET_B receptors, indicating both subtypes mediate contractions in response to ET-1. Administration of the NO-donor nitroglycerin, counteracted these contractions for the most part, confirming that NO is able to neutralize the effects of ET-1 during physiological conditions.[68, 74] The concentration at which ET-1 provokes a vasocontractile response in these experimental models is higher than its *in vivo* plasma levels.[68, 70, 71] Thus, consistent with a predominantly abluminal release of ET-1, local production of ET-1 in the placenta determines the vasopressor responses to this peptide. The potency of ET-1 is maintained throughout the fetoplacental vascular tree,[71] unlike those of serotonin and thromboxane A_2 (TxA_2), which decline dramatically when reaching stem villous arteries, making ET-1 an important substance in maintaining placental vascular resistance.[71, 75]

A role for ETs has been proposed in cardiovascular disease including hypertension.[76] Moreover, activation of the ET system has been implicated as a key final pathway in the pathogenesis of PE. Indeed, most studies have reported elevated plasma ET-1 levels in women with PE as compared to normotensive pregnant women, while some studies even demonstrate a positive correlation with disease severity.[66, 77-79] Benoit *et al.* investigated the vasoconstrictive function of ET-1 in PE placentas by exposing healthy isolated chorionic plate arteries to conditioned medium prepared from PE placentas. In their study, enhanced vasoconstriction was shown in response to this medium, which was in part inhibited by an ET_A receptor blocker, while no effect was seen when blocking the ET_B receptor.[80] The latter finding suggests that an altered response to ET-1 is not the major contributor to the elevated vasoconstriction observed in PE. In keeping with these findings, both in the *ex vivo* placental perfusion model and in isolated placental arteries, no differences were observed in vascular response to ET-1 between healthy and PE placentas.[81, 82] Nevertheless, elevated levels of ET-1 in the placenta may still account for increased placental vascular resistance *in vivo* and could further play a part in the maternal endothelial dysfunction observed in PE. Indeed, as discussed earlier, women with PE display elevated circulating concentrations of ET-1. Similarly, increased ET-1 mRNA expression has been observed in placental tissue[83, 84] of PE pregnancies. Thus, the main question that needs to be answered is: what causes this uniform rise in ET-1 in women with PE? One prevailing theory is that elevated placental ET-1 production is released into the maternal circulation and triggers the manifestations of PE. The augmented release of this vasoactive peptide could be triggered by placenta-derived factors resulting from placental ischemia. In preclinical studies, pregnant mice infused with tumor necrosis factor-α (TNF-α) or AT_1R-AA display significantly increased expression of preproET-1 in the placenta, and in the case of TNF-α, also in the maternal aorta.[60] In addition, the sFlt-1 concentration is positively correlated with a rise in ET-1 plasma levels.[43] Intriguingly, administration of an ET_A receptor antagonist in sFlt-1-infused mice significantly abolished hypertension.[37] Hence, it is possible that placental sFlt-1 directly induces local ET-1 production. Experimental animal studies were not able to confirm this theory, as exogenous administration of sFlt-1 in pregnant mice only induced preproET-1 mRNA levels in the kidney but not in the placenta.[37] The observation that treatment with VEGF inhibitors in cancer patients or rats induces a PE-like syndrome with a rise in ET-1,[38] further indicates that the ET-1 elevation in the maternal circulation is a direct consequence of VEGF inactivation by sFlt-1.[59] Taken together, it is plausible to assume that the elevated sFlt-1 levels rather than local ET-1 production in the placenta are the main source of the increased circulating ET-1 levels in PE. Since ET-1 triggers oxidative stress in placental tissue,[85] a vicious cycle may arise, where ET-1 production in the placenta contributes to the release of placenta-derived factors including sFlt-1 into the maternal circulation.

This hypothesis, which suggests that placental ET-1 overproduction may contribute to enhanced release of sFlt-1 into the maternal circulation, was recently investigated by Li *et al.,* making use of mice with a modified ET-1 gene that causes high ET-1 production.[86] Their study demonstrates that maternal overproduction of ET-1 in pregnant mice is responsible for the development of full spectrum PE-like symptoms. However, placental overproduction of ET-1 was significantly associated with high plasma sFlt-1 levels in the maternal circulation, and contributed to elevated blood pressure. These findings confirm the concept that local ET-1 production in the placenta could contribute to PE through increasing sFlt-1 release in the maternal circulation. In turn, as discussed, sFlt-1 binds to VEGF, thereby inducing endothelial dysfunction and increasing plasma ET-1 production. Additionally, the decreased circulating volume and decreased placental perfusion[59] in PE women might further elevate placental ET-1 levels. Alterations in the expression or localization of ET-1 receptors in the placenta may also enhance placental vascular resistance in PE. Rutherford *et al.*, using affinity binding assays, observed no difference in either density or affinity of the ET-1 receptors in PE placentas.[61] Faxen *et al.* reported reduced ET_A receptor mRNA expression in PE placentas, while in another study this was only observed in late-onset PE. Downregulation of these receptors due to elevated ET-1 levels may underlie these findings.[87, 88] On the other hand, studies investigating ET_B receptor expression reported both increased and decreased mRNA levels in PE placentas.[88, 89] Altogether, the evidence regarding changes in placental ET receptor expression during PE remains ambiguous. Lastly, it has been hypothesized that MMPs, enzymes that can cleave bigET-1 into ET-1, are significantly increased in women with PE.[60] An elevated expression of MMP-2 in placental tissue, which has been reported recently, could account for increased ET-1 production in the placenta.[90] Also, increased ECE levels in serum of women with PE has been observed,[91] although its expression level in PE placentas has not yet been determined.

Endothelin receptor antagonists (ERAs) are clinically implemented for the treatment of cardiovascular diseases such as pulmonary hypertension, renal failure and cancer.[60] Concerning their actions in the treatment of the maternal syndrome of PE, most of our understanding comes from experimental animal models of PE. Particularly in the reduced uterine perfusion pressure (RUPP) model or sFlt-1-induced hypertension in rats, selective blockade of the ET_A receptor resulted in attenuated hypertension.[37, 92] In spite of these reports, ERAs are contraindicated in pregnancy, as genetic ET_A knockout models or treatment with ET_A receptor antagonists have led to fetal malformations or death in rodents.[93] Thaete *et al.* determined the levels of a selective ET_A receptor antagonist in fetal plasma of pregnant rats following long-term maternal exposure, and observed that only 2% of plasma levels reached the fetus.[94] Despite these findings, it is currently unknown whether ERAs cross the placental barrier in humans. Given the low molecular weight and lipophilic characteristics of these drugs, (partial) placental transfer seems

probable, since it is long known that lipophilic drugs with a molecular weight below 600 in general rapidly cross the placental barrier.[95] However, specific knowledge on ERAs in the human placenta is imperative to determine their usefulness in reducing placental vascular resistance in PE, because if ERAs are indeed to pass the placental barrier in humans, their potential teratogenic effects would possibly compromise their use in pregnancy. Linking ERAs to elastin-like peptides that do not cross this barrier, could provide an alternative approach to target the ET-1 pathway in the maternal circulation without affecting the fetus.[60] In addition, as observed by Thaete *et al.*,[94] administration of ERAs during late pregnancy, after completion of fetal organogenesis, might be harmless. Which ET-receptor subtype should be blocked requires further investigation, as few studies have investigated the role of ERAs in PE placental vessels. In healthy placental arteries, ET_A receptor antagonism and to a lesser degree ET_B receptor antagonism both diminished the vasoconstrictor response of ET-1.[68] Obviously, changes in receptor expression may influence the response to ERAs during PE.

In conclusion, the ET system plays seems to play an important role in the pathogenesis of PE. Compared to healthy pregnancy, increased circulating levels of ET-1 are found in PE. To target this system, future studies making use of the *ex vivo* human placental perfusion model should first determine the passage of ERAs through the human placental barrier. In addition, alternative mechanisms to target the ET system such as inhibiting ET-1 release, inactivating ET-1 in the circulation or suppressing endothelial ET-1 synthesis either directly (e.g with small interfering RNA) or indirectly (through inhibition of sFlt-1) should be further explored in future studies.

RENIN-ANGIOTENSIN SYSTEM

The RAS contributes to the long-term regulation of arterial pressure via its effects on vascular tone and sodium homeostasis. Angiotensinogen, the precursor to all angiotensins, is converted via renin into Ang I. Ang I is not biologically active and is cleaved primarily by angiotensin-converting enzyme (ACE) to form Ang II, the main effector peptide of the RAS. Of note, Ang II can also be generated via chymase which is produced by villous syncytiotrophoblasts, although evidence that this occurs *in vivo* is lacking.[96] Once formed, Ang II can stimulate both the angiotensin II type 1 receptor (AT_1R) and the angiotensin II type 2 receptor (AT_2R) (Figure 2). Although the affinity of Ang II is ~15-fold greater for the AT_2R than the AT_1R,[97] the AT_1R is the dominant receptor expressed following fetal life.[98] Activation of the AT_1R elicits the classical actions of the RAS including aldosterone release, sodium retention, vasoconstriction and pro-inflammatory effects. Conversely, activation of the AT_2R induces natriuresis, vasodilation and anti-inflammatory effects (Figure 2). The discovery of novel angiotensin fragments and receptors has led

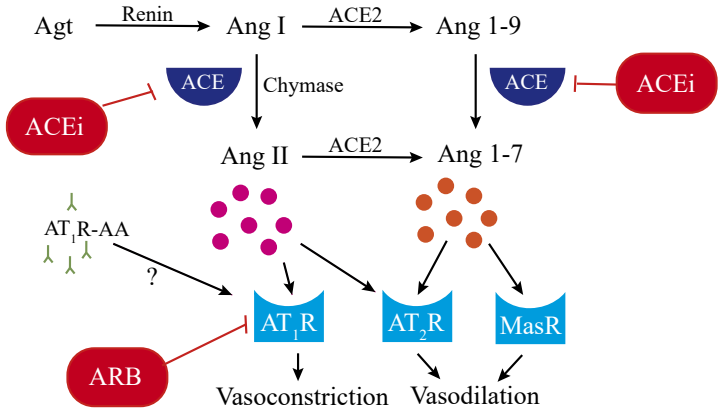

Figure 2. The RAS in human pregnancy and therapeutic strategies targeting this pathway. During PE there is a decrease in renin, Ang I, Ang II and Ang 1-7, whereas circulating levels of AT_1R-AA are increased. Abbreviations: ACE = angiotensin converting enzyme; ACEi = ACE inhibitor; Agt = angiotensinogen; Ang = angiotensin; ARB = angiotensin II type 1 receptor blocker; AT_1R = angiotensin II type 1 receptor; AT_2R angiotensin II type 2 receptor; AT_1R-AA; angiotensin II type 1 auto-antibodies; MasR = Mas receptor.

to the identification of depressor RAS pathways, which encompasses the AT_2R and the angiotensin-converting enzyme 2 (ACE2)/Ang 1-7/Mas receptor axis, that counteract the classical actions of AT_1R stimulation.[98]

The maternal RAS undergoes major changes during pregnancy. All of the components of the RAS are increased in uncomplicated pregnancies, except for ACE.[99] During early gestation, the ovaries and maternal decidua contribute to an increase in extra-renal renin release.[100] In addition, the high estrogen level maintained by the placenta promotes hepatic angiotensinogen synthesis. Consequently, circulating Ang II and aldosterone levels are increased during pregnancy.[101] Yet, despite the increase in circulating Ang II, pregnant women and gravid animals are less sensitive to the pressor effects of Ang II.[102, 103] In a landmark study by Gant *et al.* from 1973, it was demonstrated that normotensive pregnant women require twice the dose of Ang II to elicit a 20 mmHg increase in diastolic arterial pressure as compared to non-pregnant women.[104] This refractoriness to Ang II has been attributed to increases in progesterone and PGI_2 which can decrease sensitivity to Ang II-induced vasoconstriction,[105] but may also be, at least in part, mediated by an upregulation of the depressor RAS pathways. For example, it has been demonstrated that during normotensive pregnancies circulating ACE2 is upregulated,[103, 106] plasma Ang 1-7 increases by 20-fold[103] and that the AT_2R/AT_1R balance rises.[103] Similarly, in murine studies, genetic and/or pharmacological AT_2R[107-110] or ACE2[111] deficiency leads to pregnancy induced hypertension, supporting a key role for the depressor RAS pathways in the normal cardiovascular adaptations to pregnancy.

Compared to the characteristic refractoriness to Ang II observed during normal pregnancy, PE is characterized by increased sensitivity to Ang II and altered expression of RAS components.[102, 104, 112, 113] Circulating renin, Ang I, Ang II and aldosterone levels are lower in women with PE as compared to women with uncomplicated pregnancies.[99, 114] Moreover, the pregnancy-induced increase in plasma Ang 1-7 in women with uncomplicated pregnancies is not observed during PE.[99] Although little is known about AT_2R expression during PE, women with a history of PE have a lower AT_2R/AT_1R ratio than women who had uncomplicated pregnancies,[115] suggesting that AT_2R expression is decreased during PE. Conversely, during PE, the presence of AT_1R-AA[116] and the increase in AT_1R-bradykinin receptor (B2) heterodimers[117, 118] potentiate the pro-hypertensive effects of the AT_1R. Moreover, in PE women, AT_1R-AA titer is proportional to sFlt-1, suggesting that AT_1R-AA may stimulate additional pathways (for example ET-1 and anti-angiogenetic factors (sFlt-1 and soluble endoglin (sEng)) in addition to directly activating the AT_1R.[119]

Within the placenta, all of the components necessary for a functional RAS are expressed on both the maternal and fetal sides.[120-128] The AT_1R is the predominant AT receptor expressed throughout the placenta.[120] Within the placental villi, it has been demonstrated that AT_1R are localized in and around the blood vessels.[120] Consequently, Ang II induces a potent dose-dependent vasoconstrictor effect within the fetoplacental circulation,[129-131] with isolated chorionic plate vessels being more sensitive to Ang II than segments from umbilical[132, 133] and uterine[131] arteries. Similarly, in animals, the infusion of Ang II markedly increases fetal-placental pressure.[134, 135] Using the dually perfused *ex vivo* human placenta model, Glance *et al.* demonstrated that administration of Ang II to the maternal circulation induces a dose-related increase in fetal perfusion pressure.[130] Conversely, administration of Ang II to the fetal circulation does not induce an increase in maternal perfusion pressure,[130] indicating one-way direction (maternal to fetal) of Ang II. This suggests that high circulating maternal Ang II concentration could result in elevated fetoplacental Ang II concentration and enhanced pressor responsiveness to Ang II. Moreover, sensitivity of the fetoplacental vasculature to Ang II may also be dependent on thromboxanes and/or prostaglandins since selective cyclooxygenase (COX)-1 inhibition with low dose acetylsalicylic acid (via infusion into the intervillous space)[136] or dual COX-1 and COX-2 inhibition with meclofenamate[137] attenuates Ang II-induced vasoconstriction. Interestingly, refractoriness to Ang II-induced vasoconstriction is even greater in the fetoplacental circulation than the maternal systemic circulation during late gestation.[138] Furthermore, tachyphylaxis to Ang II has been reported in the fetoplacental circulation of term placentas from uncomplicated pregnancies.[139] This reduced sensitivity to Ang II may serve to protect the fetoplacental circulation from the normally occurring increases in maternal Ang II concentration.

In contrast to uncomplicated pregnancies, angiotensinogen, Ang II and AT_1R are significantly higher in chorionic villous tissue from nulliparous third trimester PE pregnan-

cies.[140] While these authors did not observe any differences in placental ACE, ACE2, or Ang 1-7 levels between PE and uncomplicated pregnancies, there is evidence that ACE expression and activity[141] and chymase[96] are upregulated in PE placentas, which would result in an increase in placental Ang II generation. In agreement with these studies, when isolated chorionic plate arteries from uncomplicated pregnancies are perfused with placental conditioned media from PE placentas, vasoconstriction (equal to 50% of the response to KCl 100) is blunted by 40% by chymase inhibition and 20% by ACE inhibition.[80] Moreover, AT_1R blockade with losartan attenuated the contractile response to PE-conditioned media by 30%, while AT_2R blockade with PD123319 only reduced the contractile response by 16%.[80] Collectively these data suggest that elevated Ang II acting via the AT_1R may favor vasoconstriction in placental chorionic villi during PE, which may contribute to impaired fetal blood flow and poor fetal outcomes. However, to date, studies using isolated chorionic vessels or the dually perfused *ex vivo* placental perfusion model to investigate Ang II sensitivity in PE placentas have reported that the pressor response to Ang II is decreased[133, 142] or unchanged[143] as compared to placentas from uncomplicated pregnancies. The reason for the discrepancy between these studies is unclear, but may relate to the use of magnesium sulfate ($MgSO_4$) or the combined use of cesarean and vaginally delivered placentas. Previous studies have demonstrated that $MgSO_4$, which is used clinically to prevent eclampsia, attenuates the sensitivity of the fetal-placental vasculature to Ang II.[143, 144] Differential expression of RAS components in PE and uncomplicated placentas has not been observed in vaginally delivered placentas,[145] suggesting that the expression of the RAS is altered in placentas during labor.

Drugs that inhibit the RAS (ACE inhibitors (ACEi) and AT_1R blockers (ARBs)), which are a mainstay for the treatment of hypertension, are contraindicated during pregnancy due to the high risk of teratogenic effects. In preclinical studies, ACEi use was associated with increased risk of intrauterine death and still births.[146, 147] In women, ACEi during second and third trimesters of pregnancy has been shown to have adverse effects on the fetus,[148] including FGR, respiratory, renal and circulatory abnormalities, and patent ductus arteriosus.[149-151] Similar fetal abnormalities are seen with maternal ARB treatment during the second and third trimesters of pregnancy.[152] An alternative approach to antagonize the effects of AT_1R activation during PE might be to antagonize AT_1R-AA with the help of a high affinity 7-amino acid sequence ('n7AAc'). Cunningham *et al.*, have recently demonstrated that in the reduced uterine perfusion pressure model of PE, AT_1R-AA blockade with this inhibitory peptide prevents PE symptoms, including the normalization of arterial pressure and reduces sFlt-1, preproET-1, pro-inflammatory and generation of reactive oxygen species.[153] However, there is still doubt whether AT_1R-AA truly activate AT_1R *in vivo*,[154] and thus it cannot be excluded that inhibiting AT_1R-AA has effects that are unrelated to the RAS.

Taken together, in pregnancies complicated by PE the RAS is altered, featuring an increased sensitivity to Ang II and suppressed maternal plasma levels of most RAS components. Yet, the levels of angiotensinogen and Ang II in the placenta are increased. Unfortunately, drugs that directly inhibit the RAS are contraindicated in pregnancy. New approaches, such as AT_1R-AA antagonism, could be of therapeutic value in the future, once it has been established what exactly such treatment interferes with.

PROSTAGLANDINS

Two of the most important prostaglandins in pregnancy are TxA_2 and PGI_2. They both originate from the common precursor prostaglandin H_2 (PGH_2), which is formed by conversion of arachidonic acid under the influence of COX-1 and/or COX-2.[155] To form TxA_2 and PGI_2, which act as each other's natural antagonist, PGH_2 is converted by thromboxane synthase or prostacyclin synthase respectively (Figure 3). PGI_2 is a vasodilator, and a very potent inhibitor of platelet aggregation.[156] Furthermore, in pregnancy PGI_2 inhibits uterine contractility.[157] In contrast, TxA_2 is a potent vasoconstrictor, which additionally induces platelet aggregation and stimulates uterine contractility.[156, 157] The placenta has been shown to produce both TxA_2 and PGI_2, as well as prostaglandins E_2 and F_{2a}, which both exert vasoconstrictor effects in the fetoplacental vasculature (Figure 3).[131, 158-161] TxA_2 might also mediate the constrictor effects of ET-1, 5-hydroxytryptamine (5-HT) and Ang II.[162]

Compared to the non-pregnant state, serum concentrations of PGI_2 metabolites are increased during normal pregnancy, with a peak in the first trimester.[163] In PE, the production of PGI_2 in the placenta and umbilical vessels is reduced,[164, 165] while the levels of PGI_2 and its metabolites are also diminished in plasma, urine and amniotic fluid.[163, 166, 167] This indicates a possible role for decreased PGI_2 activity in the pathogenesis of PE. Remarkably, the response of the *ex vivo* perfused PE placenta to PGI_2 is diminished compared to healthy controls.[142] As one of the first, Walsh *et al.* found that there is an imbalance between TxA_2 and PGI_2 in the PE placenta, with a three-fold increase in TxA_2 production, and a 50% reduction in PGI_2 release.[168] This TxA_2/PGI_2 imbalance causes a shift towards vasoconstriction, possibly leading to hypertension and reduced uterine flow. A factor that possibly contributes to the TxA_2/PGI_2 imbalance in PE is an elevation in circulating levels of lipid peroxides.[169] Lipid peroxides stimulate TxA_2 production, and simultaneously inhibit PGI_2.[170] Wang *et al.* showed that placental perfusion with peroxides indeed leads to a rise in the TxA_2/PGI_2 ratio.[171] Trophoblast cells are the main source of this increased TxA_2 synthesis, and accordingly, they display increased expression of COX-1 in PE,[172, 173] which may further amplify the elevation of the TxA_2 plasma levels are elevated in severe PE versus normal pregnancy.[174]

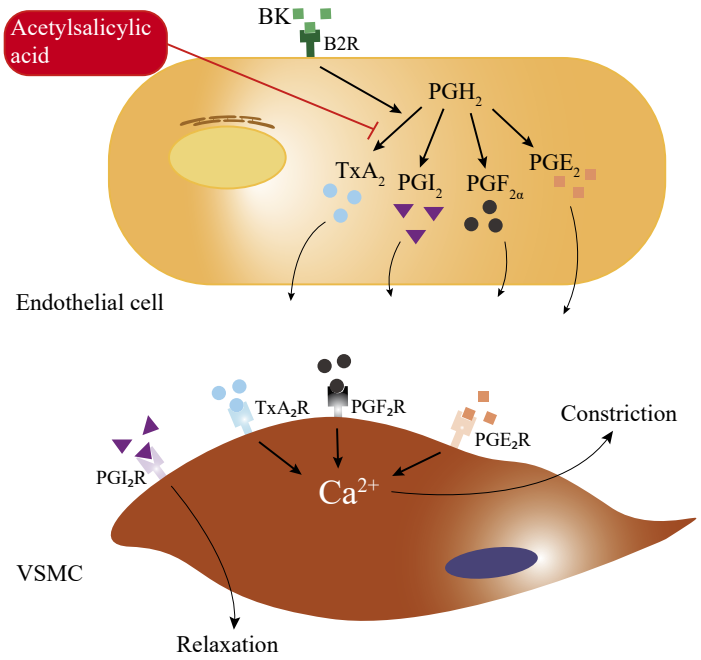

Figure 3. The prostaglandin pathway in the human placenta and therapeutic strategies targeting this pathway. During PE circulating levels of TxA_2 and $PGF_{2\alpha}$ are increased, while PGI_2 levels are decreased, resulting in an imbalance. Abbreviations: B2R = bradykinin receptor; BK = bradykinin; PGE_2 = prostaglandin E_2; PGE_2R = PGE_2 receptor; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PGF_2R = $PGF_{2\alpha}$ receptor; PGH_2 = prostaglandin H_2; PGI_2 = prostacyclin; PGI_2R = PGI_2 receptor; TxA_2 = thromboxane A_2; TxA_2R = TxA_2 receptor; VSMC = vascular smooth muscle cell.

Of all prostaglandins, TxA_2 exerts the most powerful vasoconstrictive effect in the placenta, as shown by many *ex vivo* studies.[131, 175] Administration of the TxA_2 agonist U46619 in the isolated perfused cotyledon or chorionic plate arteries elicits a potent dose-dependent response.[176-182] However, the response differs greatly among different vessel types, in contrast to other prostaglandins (e.g. E_2 and $F_{2\alpha}$) that are also known to constrict fetoplacental vasculature through binding different receptors.[75, 131] Broegger *et al.* found a positive correlation between vessel sensitivity to the TxA_2 agonist U46619 and the inner vessel diameter,[75] suggesting differences in receptor numbers and/or distribution along placental vasculature, however this still needs to be confirmed through quantification methods. These findings critically question the general opinion that TxA_2 is one of the most important regulators of placental vascular tone, since the smallest vessels are presumed to be the most contributory to resistance.[183] In PE placentas, the release of TxA_2 in response to 5-HT exposure during *ex vivo* placental perfusion is increased.[184] However, the vasoconstrictive response to U46619 is significantly attenuated,[82, 142] possibly to compensate for the higher circulating levels of TxA_2 during PE.

Targeting the TxA_2/PGI_2 imbalance as a preventive strategy for PE has been performed for a long time by studying the effects of low-dose acetylsalicylic acid. Low-dose acetylsalicylic acid selectively inhibits the synthesis of TxA_2, without interfering with the production of PGI_2.[185] This is thought to be the due to compartmentalization of the production sites of TxA_2 and PGI_2 in the placenta, as TxA_2 is primarily produced by trophoblast cells near the maternal circulation and PGI_2 by endothelial cells near the fetal circulation.[185] Studies on the efficacy of low-dose acetylsalicylic acid have shown contradictory results. Some of these differences could be explained by timing of treatment initiation or patient selection. A systematic review and meta-analysis including 10 randomized controlled trials comparing first trimester low-dose acetylsalicylic acid versus placebo or no treatment in women at risk for PE found a significant reduction in the overall risk ratio (RR 0.35; 95% CI 0.13-0.94) of developing early onset PE.[186] However, early identification of women who are at high risk for PE remains very difficult, and when started after 16 weeks of gestation the beneficial effects of acetylsalicylic acid seem no longer evident.[187, 188] Acetylsalicylic acid use is regarded safe throughout pregnancy, as no associations with adverse fetal outcome (e.g. congenital abnormalities, intraventricular hemorrhage, premature closure of the ductus arteriosus) or maternal outcome (e.g. postpartum hemorrhage, placental abruption) have been found.[189, 190] Although an increased risk of vaginal bleeding has been reported, this was not associated with an increased risk of pregnancy loss.[189] Another interesting method could be to selectively target COX-2. Sones *et al*. showed upregulation of COX-2 in a PE mouse model. Treating these mice with a single dose of the selective COX-2 inhibitor celecoxib during decidualization prevented maternal hypertension and normalized placental- and fetal growth.[191] However, the effects on PE in human pregnancy remain to be investigated. Moreover, although short term exposure to celecoxib in the third trimester showed no increase in maternal or neonatal complications,[192] the risks of long-term exposure during pregnancy are not yet known.

In conclusion, there is a distinct imbalance between TxA_2 and PGI_2 in PE, causing a preponderance towards the vasoconstrictive function of the prostaglandin axis. Until now, targeting this axis has been only proven useful in the prevention of PE in high-risk patients.

NITRIC OXIDE

NO, previously identified as endothelium-derived relaxing factor,[193] plays a key-role in vasodilation. It is synthesized by a family of nitric oxide synthases (NOS), most importantly endothelial NOS (eNOS) and inducible NOS (iNOS), that are present in various cell types including endothelial cells and fetal trophoblasts. These NOS enzymes cause ca-

talysis of L-arginine, resulting in production of NO, after stimulation by different factors (e.g. endothelial shear stress, estrogen, bradykinin, acetylcholine). By activating soluble guanylate cyclase (sGC), NO causes an increase in intracellular free cyclic guanosine 3',5'-monophosphate (cGMP), leading to vasodilation through closure of Ca^{2+} channels (Figure 4).[194-196]

In normal pregnancy, circulating levels of NO are increased,[197] as well as the blood plasma levels of hormones and growth factors that stimulate NO release, like estrogen, VEGF and PlGF.[198, 199] Apart from blood pressure regulation, NO also influences cytotrophoblast invasion and spiral artery remodeling in early gestation.[200] It has been shown in *ex vivo* placental perfusion experiments that inhibition of the NO pathway causes an increased response to the TxA_2 agonist U46619,[201] indicating that there is NO release in response to vasoconstriction, possibly due to increased shear stress.

The role of the NO pathway in PE has been extensively discussed in literature, and the mechanisms contributing to its dysfunction seem multifactorial.[194, 195] In animal models, systemic inhibition of NO synthesis leads to development of a PE-like syndrome, with maternal hypertension and proteinuria, as well as fetal growth restriction.[202, 203] Furthermore, studies using rat models with RUPP, causing PE symptoms, showed a decrease in NO synthesis and/or release from endothelial cells.[204] Altogether, these studies suggest that indeed loss of NO can contribute to development of PE. In women with PE, circulating NO metabolites have been found to be significantly decreased versus healthy pregnant women.[197, 205-207] Similarly, lower circulating levels of the NO-dependent vasodilators estrogen, VEGF and PlGF have been reported.[36, 208, 209] In addition, plasma levels of asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, are elevated in PE patients,[210] further contributing to impaired eNOS activity. Protein expression of eNOS seems unchanged in placental tissue of PE pregnancies,[206, 211] although eNOS mRNA expression has been reported to be increased, possibly as a compensatory mechanism.[212] Oxidants, such as reactive oxygen species (ROS), are produced during oxidative stress and are known to scavenge NO, resulting in the formation of peroxynitrite (Figure 4). It is thought that in PE there is an increase in ROS, explaining, at least partly, the reduced bioavailability of NO despite increased eNOS expression.[196] Indeed, peroxynitrite has been shown to be increased in vessels of women with PE.[213] Accordingly, Bisseling *et al.* found that the antioxidant N-acetylcysteine improved the NO-mediated effects in the fetoplacental circulation during *ex vivo* placental perfusion in healthy and PE placentas to a similar extent.[196] However, in a randomized controlled trial where women with severe early onset PE received either N-acetylcysteine or placebo, no improvement of maternal disease and no differences in neonatal outcome were observed.[214] Other *ex vivo* placental perfusion studies showed that administering N(ω)-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, induced a significant increase in the baseline perfusion pressure of healthy placentas.[196, 215, 216] However, in PE placentas this pressure increase

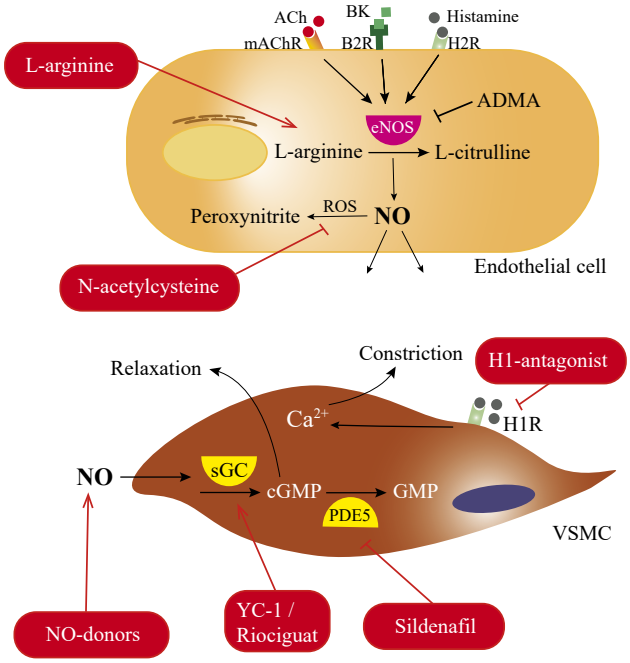

Figure 4. The NO-pathway and NO-dependent vasodilators in the human placenta and therapeutic strategies targeting this pathway. During PE there is a decrease of NO and ACh, while levels of ROS, peroxynitrite, ADMA and histamine are increased. Abbreviations: ACh = acetylcholine; ADMA = asymmetric dimethylarginine; B2R = bradykinin receptor; BK = bradykinin; cGMP = cyclic guanosine 3′,5′-monophosphate; eNOS = endothelial nitric oxide synthase; GMP = guanosine 3′,5′-monophosphate; H1R = histamine type 1 receptor; H2R = histamine type 2 receptor; mAChR = muscarinic acetylcholine receptor; NO = nitric oxide; PDE5 = phosphodiesterase-5; ROS = radical oxygen species; sGC = soluble guanylate cyclase; VSMC = vascular smooth muscle cell.

is much less pronounced, suggesting that NO-mediated vasodilation is decreased in PE.[196, 216] Another intriguing possibility is that placental eNOS is uncoupled in PE, and produces superoxide rather than NO. However, to our knowledge, this has not been investigated to date.

Many clinical trials have evaluated whether drugs that affect the NO pathway, like NO donors (e.g. organic nitrates and *S*-nitrosothiols) and NO precursors (e.g. L-arginine) are capable of restoring the NO-pathway in PE. Although they caused a significant decrease in blood pressure, there were no effects on maternal or fetal outcome.[217-221] Therefore, evidence for their effectiveness is limited, and they are additionally known to quickly develop drug tolerance and to have significant side-effects.[222] Indeed, the vasodilator response to various NO-donors is attenuated in PE placentas.[142] Studies investigating the reactivity of isolated chorionic plate arteries showed similar results. In this set-up, NO-donors result in potent vasodilation of pre-constricted healthy placental arteries,[179, 223, 224] an effect that is significantly reduced in vessels of PE placentas.[225] This lack of

efficacy of NO-donors could be due to increased scavenging of NO and/or disruptions more downstream in the NO-pathway. Interestingly, not much is known about directly modulating sGC, through either activators (e.g. YC-1) or stimulators (e.g. riociguat), in the placenta. These compounds potentiate the effects of the NO-pathway in an NO-independent way.[226, 227] Brownfoot *et al.* showed that YC-1 inhibits placental production of sFlt-1 and sEng, and that it reduces endothelial dysfunction in preeclamptic tissue.[228] Until now, no (pre)clinical trials with sGC-modulators in PE have been published, but it could be a novel therapeutic target. Another way to potentiate the action of the NO-pathway, is to inhibit the degradation of the active cGMP into the inactive GMP by phosphodiesterases. A drug that is currently under investigation is the phosphodiesterase-5 (PDE5) inhibitor sildenafil. In PE animal models, sildenafil improved fetal outcome and additionally diminished maternal symptoms.[229, 230] *Ex vivo* studies showed a significant vasodilator response of chorionic plate arteries to sildenafil.[231, 232] However, this has not been studied in placentas from PE pregnancies. Samangaya *et al.* performed a small randomized controlled trial of sildenafil versus placebo in women with PE, but found no improvement in duration of pregnancy or neonatal outcome.[233] Currently, a large multicenter randomized controlled trial is further evaluating the effects of sildenafil on pregnancy outcome in extreme FGR due to placental insufficiency.[234] Unfortunately, the first cohort of this study, from the United Kingdom, reported that sildenafil did not prolong pregnancy and did not improve fetal outcome.[235] Furthermore, the Dutch cohort has recently been halted since sildenafil did not show beneficial effects and there was an increase of neonatal complications in the treated group.[236]

To summarize, NO-mediated vasodilation is impaired in PE compared to normal pregnancy, possibly due to a decrease in NO synthesis and/or release, increased NOS inhibition, or increased NO scavenging by ROS. Although many drugs targeting this pathway have been evaluated in PE, none of them improved fetal outcome. Possibly direct stimulation and/or activation of sGC could provide a favorable treatment option.

NITRIC OXIDE-DEPENDENT VASODILATORS

Bradykinin

Bradykinin (BK) is known to be a powerful stimulator of the release of both NO and PGI_2 (Figure 4), inducing vasodilation and increasing vascular permeability through binding of the G-protein-coupled B2 receptors. In contrast, binding the B1 receptor, that is mostly expressed in the central nervous system in response to injury, induces typical signs of inflammation.[237]

In the placenta, the B2 receptor has been documented in decidua, placental and extravillous trophoblasts and in the fetal endothelium.[238] Although generally considered

to be a vasodilator, BK is also known to induce vasoconstriction in placental vessels, probably through the stimulation of TxA_2 production (Figure 3).[239] However, most *ex vivo* studies were unable to show responses of chorionic plate arteries to BK, possibly because it is rapidly metabolized in placental tissue.[177, 179, 225] After a single passage of the fetoplacental circulation, BK loses 98% of its biological activity,[240] indicating effective clearance of BK in placental vasculature. The most important enzyme responsible for BK degradation is ACE,[241] and indeed the effect of BK on placental vessels is potentiated in the presence of an ACEi.[242] BK stimulates cell migration and plays an important role in trophoblast invasion through the B2 receptors.[238] Consistent with the alterations in PE, there is a significant reduction of the B2 receptor gene- and protein expression, compared to healthy placentas.[243, 244] Possibly, this contributes to impaired trophoblast invasion. However, the precise role of BK in PE remains unknown and therapeutically targeting this system would be difficult, as for example ACEi are severely fetotoxic and, as outlined in section 5, also impact the RAS.[148]

Acetylcholine

Even though the placenta is a non-neuronal and non-innervated tissue, acetylcholine (ACh), as well as its synthesizing hormone choline acetyltransferase, are present in large concentrations in the human placenta.[245] ACh is continuously synthesized in the syncytiotrophoblast cells from where it is released into both the maternal and fetal circulations, as shown by *ex vivo* placental perfusion studies. However, it does not seem to have an effect on fetoplacental vascular tone.[246] ACh exerts its action through 2 types of cholinergic receptors: the muscarinic (mAChR) and nicotinic receptors (nAChR), both of which are expressed in the human placenta.[247, 248] It has been suggested that ACh plays an important role in the trans-placental passage of amino acids, and in placental hormone release.[249] Furthermore, it is involved in the development of placental vessels and syncytiotrophoblasts.[250]

In the PE placenta, ACh synthesis and output are decreased,[251, 252] as is the density of mAChR.[253] In contrast, the expression of nAChR is increased.[254] Interestingly, binding of nicotine to nAChR stimulates the production of growth factors, such as VEGF, leading to increased angiogenesis.[255] In line with this, smoking during pregnancy seems to be protective against PE,[256, 257] although it is well known that smoking increases the risk of many other adverse pregnancy outcomes. To investigate the therapeutic potential of nAChR agonists in PE, Mimura *et al.* tested the effect of nicotine on damaged endothelial cells. They found that nicotine significantly increased PlGF levels and counteracted the endothelial dysfunction caused by increased sFlt-1 levels, suggesting a possible therapeutic target for restoring the anti-angiogenic imbalance as seen in PE.[258]

Histamine

Histamine is an angiogenic and vasoactive mediator, that is derived from mast cells.[259] It plays an important role in allergic and inflammatory processes, and is also involved in pregnancy.[260] Histamine exerts its biological actions through binding to four receptors, two of which are involved in vascular reactivity: H1 and H2. Binding to the H1 receptor causes contraction of smooth muscle cell, whereas activation of the H2 receptor stimulates vasodilation (Figure 4).[260]

In first trimester, maternal serum levels of histamine are highest, decreasing with progressing gestation.[261] Histamine has been shown to promote trophoblast invasion.[262] During placental development, hypoxia is an important trigger for mast cell activation, stimulating angiogenesis through production of HIF-1α and VEGF. HIF-1α activity leads to increased synthesis of histamine within mast cells, and their degranulation.[263] Isolated vessel studies show that in human placental arteries and veins, histamine predominantly causes vasoconstriction,[264-266] an effect that is attenuated by adding a H1 receptor blocker.[264] However, a vasodilator effect on placental arteries through the H2 receptor has also been recorded.[267] Both effects indicate that histamine is involved in regulation of placental vascular tone. Histamine is predominantly metabolized by diamine oxidase (DAO), an enzyme that is highly expressed in, and produced by the placenta.[268] An increase in circulating DAO levels during advancing gestation causes a decrease of circulating histamine levels. In animal studies, histamine injection and/or DAO inhibition had fatal consequences, indicating a protective role for DAO against reaching harmful histamine levels.[269, 270] Also in human pregnancy, reduced activity of DAO has been linked to unfavorable outcome.[268]

In pregnancies complicated by PE, placental tissue concentrations of histamine and mast cells are significantly higher as compared to normal pregnancy.[271, 272] Furthermore, higher mast cell density and a lower vascular/extravascular index of histamine have been identified, indicating a change in mast cell distribution, as well as in circulating histamine concentration.[272] On the contrary, isolated placental arteries of PE placentas show a decreased responsiveness to histamine *ex vivo* indicating reduced placental sensitivity for histamine.[266] Whether blockade of the H1 receptor, or stimulation of the H2 receptor could offer treatment potential for PE has not yet been investigated.

SEROTONIN AND TRYPTOPHAN

Tryptophan (Trp) is an essential amino acid and, in addition to its requirement in protein synthesis, is the precursor to several vasoactive metabolites. Two main Trp metabolizing pathways are present in the placenta, being the serotonin pathway initiated by conversion of Trp by tryptophan hydroxylase (TPH) and the kynurenine pathway, initiated by

conversion of Trp by either tryptophan 2,3-dioxygenase (TDO), indolamine 2,3-dioxygenase-1 (IDO1), or indolamine 2,3-dioxygenase-2 (IDO2).[273]

Serotonin

Serotonin, also known as 5-HT, is mainly known for its functions in the central nervous system. Furthermore, it regulates blood vessel tone and blood pressure. For these functions outside the central nervous system 5-HT might originate from platelets.[274] Additionally, locally produced serotonin might aid in regulation of placental blood vessel tone. Serotonin is a metabolite of Trp, and the responsible enzyme, Trp hydroxylase, is expressed in the placenta.[275] In blood vessels 5-HT can exert different effects, ranging from constriction to dilation, depending on the receptor(s) it binds to.[276] Watts *et al.* concluded that 5-HT is able to induce vasoconstriction in virtually every isolated blood vessel, mediated predominantly by 5-HT2A (Figure 5), and partly by 5-HT1B/1D receptors.[276] Results from wire-myography experiments reveal that segments from umbilical and chorionic plate arteries and veins show a strong dose dependent vasoconstrictive response to 5-HT, dependent on 5-HT2 and not 5-HT1 or 5-HT3 receptors.[131, 177, 277-280]

Although placental arteries and veins respond similarly to 5-HT, sensitivity to 5-HT decreases with vessel size.[71, 132] Like with prostaglandins, this could indicate differences in receptor density and/or distribution. Given the wide variety of constrictor and dilator 5-HT receptor,[276] it is not surprising that in perfused vessel segments 5-HT often elicits a biphasic pressure response, with a pressure decrease preceding vasoconstriction.[281] In *ex vivo* placental perfusion experiments infusion with 5-HT results in a dose-dependent elevation in perfusion pressure.[82, 162, 216, 282-284] However, these rises in pressure vary greatly between experiments and often decline slowly.

Responses to 5-HT are largely dependent on calcium (Ca^{2+}). Hence, both the extracellular Ca^{2+} concentration and Ca^{2+} antagonists affect the 5-HT response in chorionic arteries and veins.[279] Other factors involved in the 5-HT response are prostaglandins, more specifically TxA_2. This prostanoid was detectable in the perfusate of *ex vivo* perfused placentas after 5-HT induced vasoconstriction, supporting the concept that placental vessels are able to produce TxA_2 locally (Figure 5). Moreover, both the COX-inhibitor acetylsalicylic acid and the TxA_2 antagonist GR32191 significantly attenuate the vasoconstriction in response to 5-HT.[162] Not only TxA_2, but also prostaglandin $F_{2\alpha}$ potentiates the 5-HT response in chorionic veins in both wire-myography and vessel segment perfusion experiments.[278] These results show that prostaglandins potentially regulate vascular resistance by modifying the 5-HT response.

Blood levels of 5-HT change during pregnancy and are affected by PE. Whereas the platelet 5-HT concentration increases during both healthy and PE pregnancies, the free 5-HT concentration is elevated during PE pregnancies only, in both the maternal and fetal circulation.[285-287] Moreover, this free 5-HT plasma concentration directly correlates

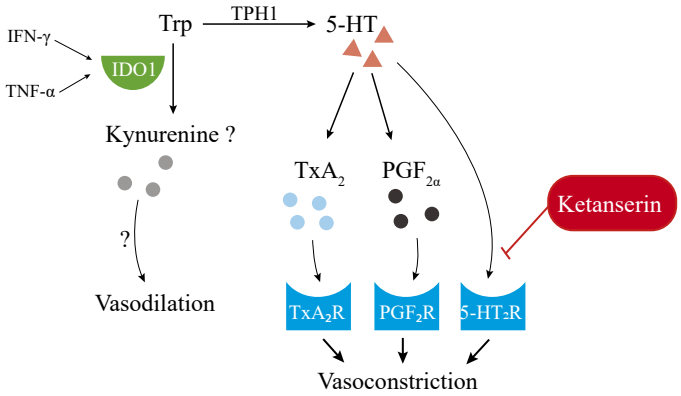

Figure 5. 5-HT and tryptophan in human pregnancy and therapeutic strategies targeting this pathway. During PE there is a decrease of IDO1, while circulating levels of TxA_2, $PGF_{2\alpha}$ and 5-HT are increased. Abbreviations: 5-HT = 5-hydroxytryptamine; 5-HT2R = 5-HT type 2 receptor; IDO = indolamine 2,3-dioxygenase; IFN-γ = interferon-γ; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PGF2R = PGF_2 receptor; TNF-α = tumor necrosis factor-α; TPH1 = tryptophan hydroxylase-1; Trp = tryptophan; TxA_2 = thromboxane A_2; TxA_2R = TxA_2 receptor.

with systolic and diastolic blood pressure in women suffering from severe PE.[287] *Ex vivo* placental perfusion experiments showed unaltered or increased vascular resistance in response to 5-HT in PE as compared to healthy placentas.[82, 184] Conversely, in isolated umbilical vessels and placental veins from PE placentas, the sensitivity to 5-HT did not increase throughout the third trimester like in healthy placentas, with a reduced 5-HT vasoconstrictor response as compared to healthy placental veins as a result.[266, 288]

Research on therapeutic possibilities of interfering with the 5-HT pathway has mainly focused on the role and safety of antidepressants such as selective serotonin reuptake inhibitors (SSRI), serotonin-norepinephrine reuptake inhibitors (SNRI), and tricyclic antidepressants. Studies have not been conclusive, possibly because PE has been defined in various ways, and effects of antidepressants might depend on the timing of exposure during pregnancy.[289] Platelets of PE patients have a higher transport rate of 5-HT compared to healthy pregnant women.[290] Importantly, since a large prospective population-based study has shown that depression per se results in an increased risk of developing PE in women suffering from depression, independently of treatment,[291] effects of antidepressants on PE should be convincingly distinguished from the effect of the depression itself. Indeed, women who took antidepressant medications, but without depression, tended to display an increase in the risk of PE.[292] It could be reasoned that inhibition of the responsible serotonin transporter by SSRI could contribute to a reduced platelet 5-HT delivery in the periphery, and prevention of undesired vasoconstriction. The use of another drug, the 5-HT2 receptor antagonist ketanserin, was aborted in a double-blind randomized trial due to persistent hypertension in severe early-onset PE, despite promising results in earlier studies.[293, 294] Out of the women that received

ketanserin, 73.3% experienced persistent hypertension, which was treated successfully with the rescue medication nicardipine.[293] Besides the disappointing effects on blood pressure reduction, the use of ketanserin could also not aid in the prevention of further complications or pregnancy prolongation.[293]

In conclusion, whereas 5-HT induced vasoconstriction is equal or potentially even increased in the microvasculature of PE placentas, larger vessels seem to be less sensitive to 5-HT compared to those in healthy pregnancy. This difference between the larger and smaller vessels might be due to the presence or absence of different 5-HT receptors, that can have opposing effects. Nonetheless, the combined increased plasma levels and increased microvascular sensitivity to 5-HT can have a detrimental effect on vascular resistance *in vivo* and thus complicate placental blood flow and cause progressive worsening of PE.

Tryptophan

Trp conversion occurs for 95% via the kynurenine pathway into kynurenine and other metabolites (Figure 5). These metabolites have functions in inflammation and regulation of vascular tone.[295] The first and rate-limiting step of the pathway is the conversion of Trp to N-formylkynurenine, by either TDO, IDO1, or IDO2. While TDO is mainly expressed in the liver, IDO1 is highly expressed in the placenta during pregnancy. Its expression increases with the duration of pregnancy, and in closer proximity of the fetal-maternal interface.[296] Despite several conflicting reports, cell types in which IDO1 seems to be present include trophoblasts and decidual immune- and stromal cells.[297] In placental tissue, the microvascular endothelial cells together with the ends of maternal spiral arteries are the main locations of IDO1 expression.[298, 299] The high IDO1 expression is generally believed to be essential for maintenance of fetal-maternal tolerance.[297, 300] However, studies in animals have shown that Trp metabolism via IDO1 might be involved in regulation of vascular tone as well.[301] Knowledge on the effects of Trp in the human placental vasculature is very limited. A recent study showed that Trp can induce vascular relaxation in isolated vessel segments of the chorionic plate arteries. IDO1 seems to play a critical role in this process, since its inhibition attenuates the vascular relaxation in the presence of Trp. However, for isolated chorionic plate arteries to relax in response to Trp, it was necessary to stimulate them overnight with TNF-α and interferon (IFN)-γ,[302] which might be due to the fact that chorionic plate arteries do not express IDO1 under normal circumstances[298] and therefore, it needs to be upregulated by these inflammatory cytokines first before an effect can be seen. The *ex vivo* perfused cotyledon responded to Trp with a decrease in fetal perfusion pressure, even without pre-constriction of the placental vasculature,[302] suggesting that IDO1 metabolites contribute to regulation of placental microvascular resistance vessels. Since microvascular endothelial cells of the placenta were reported to express IDO1 under normal physiological conditions, it is

likely that these cells contribute to the vascular relaxation in response to Trp.[298] IDO1 is also present in vascular endothelial cells of the decidua basalis,[298] and thus it could be speculated that endothelial IDO1 has a vasodilatory effect in the maternal decidua. Trp is an endothelium dependent vasodilator, and the metabolite kynurenine is proposed to contribute to this vasodilator response (Figure 5).[301, 303] However, there might also be other metabolites with vasodilator effects besides kynurenine. Although these exact metabolites and mechanisms that contribute to vasodilation remain to be identified, the metabolism of Trp by IDO1 plays an important role in the regulation of vascular tone in placental microvasculature.

Altered Trp metabolism through the IDO1 pathway may be involved in the development and/or progression of PE and FGR. In these conditions, expression and activity of IDO1 is reduced.[299, 302, 304, 305] Yet, after overnight stimulation of chorionic arteries of PE and FGR placentas with TNF-α and IFN-γ, Trp still induced vascular relaxation.[302] However, these results should be interpreted with caution, since experiment numbers were very low and the added doses of Trp substantially exceeded physiological levels. *Ex vivo* perfusion experiments measuring the response of PE placentas to Trp in the presence and absence of IDO1 inhibitors are required to establish whether indeed IDO1 plays a critical role in PE.

If reduced Trp metabolism due to low IDO1 expression is causal for increasing placental vascular resistance, finding ways to increase Trp metabolism by IDO1 could be a potential target for treatment of the reduced placental blood flow in PE and FGR. However, it should be noted that, in contrast to PE, which is associated with reduced IDO1 activity, many other diseases are associated with increased Trp metabolism, and IDO1 inhibitors are now being tested in clinical trials as anticancer therapy. Extreme caution is warranted for use of these inhibitors during pregnancy and in women at child bearing age, as they might have detrimental effects on pregnancy progression.

Altogether, the metabolism of Trp by IDO1 seems to be crucial for a healthy pregnancy progression. The reported functions of Trp metabolism in regulation of vascular tone, together with the fact that IDO1 is mainly expressed in microvascular endothelial cells, imply a role for IDO1 in regulation of vascular tone in the placenta. Finally, given the fact that 95% of Trp is metabolized through the kynurenine pathway, it is possible that even a small reduction of IDO1 in PE may substantially increase the amount of Trp that is metabolized through the serotonin pathway, which, as outline above, is more activated in PE.

CALCITONIN GENE-RELATED PEPTIDE

CGRP is a neuropeptide, widely distributed in the central and peripheral nervous system, that has a potent vasodilator effect on vascular tone. CGRP acts through binding of the G protein-coupled calcitonin receptor-like receptor (CRLR), in the presence of the receptor activity modifying protein-1 ($RAMP_1$).[306]

In the human placenta, both CRLR and $RAMP_1$ are abundantly expressed in the vascular endothelium and underlying smooth muscle cells of the umbilical, chorionic and stem villous vessels, as well as in the villous trophoblast.[307] Stevenson *et al.* showed a significant increase in maternal plasma CGRP levels throughout normal pregnancy, with a rapid drop after delivery, indicating the placenta as a source of CGRP production.[308] Furthermore, it has been shown that there is a weight- and gestational age-dependent increase in neonatal plasma CGRP levels.[309] This suggests a role for CGRP in vascular adaptation in pregnancy and fetal growth and development. It has also been suggested that CGRP is involved in maintaining uterine relaxation during pregnancy.[310] Isolated chorionic plate arteries show a dose-dependent relaxation response to CGRP, an effect that is attenuated in the presence of a CGRP receptor antagonist, further indicating that CGRP plays a role in the control of fetoplacental vascular tone.[307, 311] In placental arteries of pregnancies complicated by PE or FGR the vasodilator effect of CGRP is significantly reduced.[311-313] In line with this finding, Dong *et al.* showed that mRNA expression of CRLR and $RAMP_1$ in placental vessels of PE pregnancies is reduced, which was accompanied by a decrease in CRLR and $RAMP_1$ protein expression and CGRP binding sites in vascular and trophoblast tissue of PE placentas.[313] Furthermore, maternal serum levels of CGRP are significantly lower in women with PE compared to uncomplicated pregnancies.[314] This evidence supports a potential role of compromised CGRP-mediated vasodilation in the pathogenesis of PE. In animal models of PE, administration of CGRP reduced maternal hypertension and pup mortality in rats.[315] In agreement with this finding, infusion of a CGRP receptor antagonist induced maternal hypertension and caused a significant decrease in pup weight.[316]

Unfortunately, knowledge on the underlying mechanisms of CGRP on fetoplacental development is still lacking and direct targeting of the CGRP-pathway remains complicated. CGRP itself can only be administered parenterally, has a very short half-life and is costly. Rutaecarpine, a traditional Chinese drug that potentiates the release of endogenous CGRP, could be a therapeutic option. However, its effect in pregnancy is unknown.[317] Also, an αCGRP analogue with a longer half-life has been described in murine studies, investigating its application in cardiovascular disease, where it showed antihypertensive effects.[318]

CONCLUSION AND PERSPECTIVES

Since the blood vessels of the fetoplacental vasculature lack autonomous innervation, circulating and locally produced hormones are essential in regulating vascular tone. As has been shown in this review, mechanisms behind this regulation are very intricate, as they involve many pathways, and are influenced by numerous factors. Adequate development of the placenta is essential for an optimal course of pregnancy and subsequent maternal, fetal and neonatal outcome. The pathophysiology of placental diseases such as PE seems multi-factorial and complex, including a cascade of dysregulated systems. Finding new treatment options that safely prolong pregnancy is essential for reducing the risk of fetal, neonatal and maternal complications. This review has highlighted the importance of focusing on restoring the dysfunctional vascular regulatory systems when studying treatment strategies for PE. Techniques using human tissue, such as the *ex vivo* placental perfusion model and wire-myography, are indispensable in unraveling the vasoactive profile of the human placenta, to help understand the pathological changes occurring during PE.

Future research should focus on both efficacy and safety, by performing well designed dose-finding studies before starting clinical trials. Targeting the ET-axis by blocking the ET-1 mediated effects or restoring the sFlt-1/PlGF imbalance currently seems to have the greatest therapeutic potential. Here a first step might be to determine trans-placental passage of ERAs and to quantify their effects on sFlt-1 release in the *ex vivo* perfusion model. Another promising treatment option would be increasing NO-mediated vasodilation through sGC stimulators or activators. Although such drugs showed beneficial effects on placental tissue, results of clinical trials are still not available. When considering new drugs for treatment, the first step should be to determine their trans-placental transfer and effect on the fetal vasculature, for which the *ex vivo* placental perfusion model is a very reliable method. Parallel studies with isolated arteries may help to obtain a more detailed mechanistic insight. With this, differences in pathophysiology between early- and late onset PE and the changes in pharmacokinetics that occur during pregnancy should be kept in mind. Furthermore, individual differences in pathway disturbance may call for a more personalized therapeutic approach.

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